

Entry of the new Claims and early and favorable action on the merits of this application are respectfully solicited.

Respectfully submitted,

THE FIRM OF HUESCHEN AND SAGE

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Enclosure: Postal Card Receipt
Claims 29 through 61

We Claim:

- 29 -

The use of a membrane fraction of Gram-negative bacteria, comprising proteoglycans, for preparing a pharmaceutical composition which is immunostimulant and/or which is capable of inducing an antitumor immune response.

- 30 -

The use of Claim 29, wherein the membrane fraction comprises a membrane fraction of *Klebsiella pneumoniae*.

- 31 -

The use of Claim 29, wherein the membrane fraction comprises membrane fractions of at least two different strains of bacteria.

- 32 -

The use of Claim 29, wherein preparation of the membrane fraction comprises the following steps:

- a) culturing the bacteria in a culture medium which allows their growth, followed by centrifugation of the culture;
- b) where appropriate, deactivation of the lytic enzymes of the bacterial pellet obtained in step a), then centrifugation of the suspension obtained;
- c) extraction and elimination of the non-membrane-bound proteins and of the nucleic acids of the pellet obtained in step a) or b) with at least one cycle of washing the pellet in an extraction solution;
- d) digestion of the membrane pellet obtained in step c) in the presence of protease enzymes, followed by centrifugation;
- e) at least one cycle of washing the pellet obtained in step d) in a physiological solution and/or in distilled water; and
- f) ultrasonication of the pellet obtained in step e).

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- 33 -

The use of Claim 29, wherein preparation of the membrane fraction comprises the following steps:

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- a) culturing of the bacteria in a culture medium which allows their growth followed, where appropriate, by centrifugation;
 - b) freezing of the culture medium or of the pellet obtained in step a), followed by thawing and drying of the cells;
 - c) elimination, using a DNase, of the nucleic acids from the dried cells obtained in step b), which have been resuspended;
 - d) grinding of the cells obtained in step c) and clarification of the suspension obtained;
 - e) precipitation, in acid medium, of the suspension obtained in step d) and elimination of the pellet;
 - f) neutralization of the supernatant obtained in step e) containing the membrane suspension, followed by dialysis and concentration of the membrane suspension; and
 - g) sterilization of the concentrated membrane suspension obtained in step f).

- 34 -

The use of Claim 29, wherein the pharmaceutical composition also comprises a vehicle agent for the membrane fraction in a form which makes it possible to improve its stability and/or its immunostimulant activity and/or its capacity to induce an antitumor immune response.

- 35 -

The use of Claim 34, wherein the agent is of the oil-in-water or water-in-oil emulsion type.

- 36 -

The use of Claim 34, wherein the agent is in the form of a particle of the liposome, microsphere or nanosphere type, or any type of structure which enables said membrane fraction to be encapsulated and presented in particulate form.

- 37 -

The use of Claim 29, wherein the pharmaceutical composition also comprises an agent for potentiating the immunostimulant activity and/or the antitumor immune response of said membrane fractions.

- 38 -

The use of Claim 37, wherein the agent for potentiating the immunostimulant activity and/or the antitumor immune response of said membrane fractions is a cytokine.

- 39 -

The use of Claim 37, wherein the agent for potentiating the immunostimulant activity and/or the antitumor immune response of said membrane fractions is a regulatory agent chosen from hormones.

- 40 -

The use of Claim 37, wherein the agent for potentiating the immunostimulant activity and/or the antitumor immune response of said membrane fractions is a regulatory agent chosen from growth factors.

- 41 -

The use of Claim 37, wherein the agent for potentiating the immunostimulant activity and/or the antitumor immune response of said membrane fractions is a cellular compound.

- 42 -

The use of Claim 41, wherein the cellular compound is a nucleic acid chosen from DNAs and RNAs.

- 43 -

The use of Claim 41, wherein the cellular compound is a compound of the ribosome family.

- 44 -

The use of Claim 41, wherein the cellular compound is a protein of the heat-shock protein family.

- 45 -

The use of Claim 29 for preparing pharmaceutical composition intended to be administered in combination with an anticancer treatment.

- 46 -

The use of Claim 45, wherein the anticancer treatment is chemotherapy and/or radiotherapy.

- 47 -

The use of Claim 45 for preparing a pharmaceutical composition intended to be administered simultaneously with, separately from, or at intervals with, the anticancer treatment.

- 48 -

The use of Claim 47, wherein the pharmaceutical composition is administered enterally or parenterally.

- 49 -

The use of Claim 45, wherein the combined anticancer treatment is a chemotherapeutic treatment comprising a protease inhibitor or a compound with anti-angiogenic activity.

- 50 -

The use of Claim 29 for preventing and/or treating cancers.

- 51 -

The use of Claim 50 for preventing and/or treating bladder cancers, prostate cancers, colon cancers, liver cancers and malignant melanomas.

- 52 -

A pharmaceutical composition comprising a membrane fraction of Gram-negative bacteria, comprising proteoglycans, which can be obtained using a method for preparing a membrane fraction of Claim 32.

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- 53 -

A pharmaceutical composition comprising a membrane fraction of Gram-negative bacteria, comprising proteoglycans, which can be obtained using a method for preparing a membrane fraction of Claim 33.

- 54 -

The pharmaceutical composition of Claim 52, wherein the Gram-negative bacterium is *Klebsiella pneumoniae*.

- 55 -

The pharmaceutical composition of Claim 53, wherein the Gram-negative bacterium is *Klebsiella pneumoniae*.

- 56 -

The pharmaceutical composition of Claim 52 which is combined with an anticancer treatment by chemotherapy and/or by radiotherapy.

- 57 -

The pharmaceutical composition of Claim 53 which is combined with an anticancer treatment by chemotherapy and/or by radiotherapy.

- 58 -

The pharmaceutical composition of Claim 56 which contains an anticancer compound as a combination product for use which is simultaneous, separate, or at intervals.

- 59 -

The pharmaceutical composition of Claim 57 which contains an anticancer compound as a combination product for use which is simultaneous, separate, or at intervals.

- 60 -

The pharmaceutical composition of Claim 58, wherein the anticancer compound is chosen from protease inhibitors or from compounds with anti-angiogenic activity.

- 61 -

The pharmaceutical composition of Claim 59, wherein the anticancer compound is chosen from protease inhibitors or from compounds with anti-angiogenic activity.
